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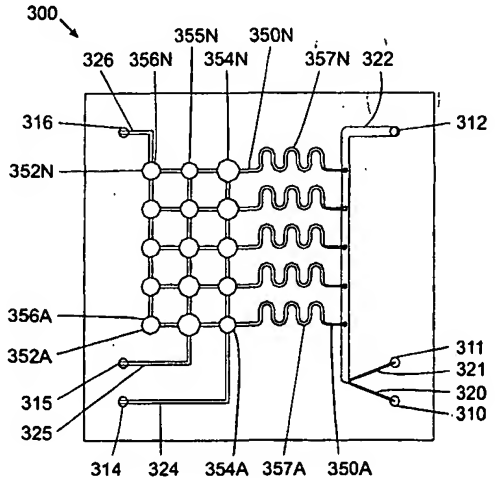
(54) Title: MICROFLUIDIC CLOSED-END METERING SYSTEMS AND METHODS



(57) Abstract: Microfluidic devices and methods for segregating aliquots of fluid from large fluid volumes are provided. Preferably, a device includes an actuating channel, a metering channel and a deformable membrane disposed therebetween. The metering channel is in fluid communication with a fluid source, but is otherwise closed. The pressure in the actuating channel may be varied to deform the deformable membrane. The volume of the metering channel varies in proportion with the deformation of the deformable membrane, creating a pressure differential between the metering channel and the fluid source that causes fluid to be drawn into or expelled from the metering channel. Magnetic or mechanical actuating means may be substituted for the actuating channel. Multiple aliquots of different liquids may be drawn into metering channels and mixed thereafter in one or many different mixing proportions.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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DESCRIPTION

Microfluidic Closed-End Metering Systems And Methods

Statement of Related Application(s)

This application claims priority to U.S. Patent Application Serial No. 10/090,092, filed July 3, 2002.

Field Of The Invention

The present invention relates to microfluidic devices and the manipulation of fluid within those devices.

Background Of The Invention

There has been a growing interest in the manufacture and use of microfluidic systems for the acquisition of chemical and biological information. In particular, when conducted in microfluidic volumes, complex chemical and biochemical reactions may be carried out using very small volumes of liquid. Among other benefits, microfluidic systems improve the response time of reactions, minimize sample volume, and reduce reagent consumption. When volatile or hazardous materials are used or generated, performing reactions in microfluidic volumes also enhances safety and reduces disposal quantities.

There exist well-recognized differences between microscopic and macroscopic flow. To begin with, fluid flow within microfluidic systems is characterized by being within the laminar, rather than turbulent, regime due to the small dimensions. In other words, one by-product of the high surface-to-volume ratios within typical microfluidic systems is that viscous effects dominate over momentum effects. Some important phenomena in microfluidics are the tendency of gas bubbles (if present) to block fluid exits, and the difficulty of mixing liquids in such systems.

Traditionally, microfluidic devices have been constructed in a planar fashion using techniques that are borrowed from the silicon fabrication industry. Representative systems are described, for example, in some early work by Manz et al. (Trends in Anal. Chem. (1990) 10(5): 144-149; Advances in Chromatography (1993) 33: 1-66). In these publications, microfluidic devices are constructed by using photolithography to define channels on silicon or glass substrates and etching techniques to remove material from the substrate to form the channels. A cover plate

is bonded to the top of the device to provide closure. Miniature pumps and valves can also be constructed to be integral (e.g., within) such devices. Alternatively, separate or off-line pumping mechanisms are contemplated.

More recently, a number of methods have been developed that allow microfluidic devices to be constructed from plastic, silicone or other polymeric materials. In one such method, a negative mold is first constructed, and plastic or silicone is then poured into or over the mold. The mold can be constructed using a silicon wafer (see, e.g., Duffy et al., Analytical Chemistry (1998) 70: 4974-4984; McCormick et. al., Analytical Chemistry (1997) 69: 2626 –2630), or by building a traditional injection molding cavity for plastic devices. Some molding facilities have developed techniques to construct extremely small molds. Components constructed using a LIGA technique have been developed at the Karolsruhe Nuclear Research center in Germany (see, e.g., Schomburg et al., Journal of Micromechanical Microengineering (1994) 4: 186-191), and commercialized by MicroParts (Dortmund, Germany). Jenoptik (Jena, Germany) also uses LIGA and a hot-embossing technique. Imprinting methods in PMMA have also been demonstrated (see, Martynova et.al., Analytical Chemistry (1997) 69: 4783-4789). However, these techniques do not lend themselves to rapid prototyping and manufacturing flexibility.

Various conventional tools and combinations of tools are used when analyzing or synthesizing chemical or biological products in conventional macroscopic volumes. Such tools include, for example: metering devices, reactors, valves, heaters, coolers, mixers, splitters, diverters, cannulas, filters, condensers, incubators, separation devices, and catalyst devices. Attempts to perform chemical or biological synthesis and/or analysis in microfluidic volumes have been stifled by difficulties in making tools for analysis and/or synthesis at microfluidic scale and then integrating such tools into microfluidic devices. Another difficulty is accurately measuring stoichiometric microfluidic volumes of reagents and solvents to perform analysis and/or synthesis on a microfluidic scale. Additionally, difficulties in rapidly prototypic microfluidic devices are compounded by attempts to incorporate multiple analysis and/or synthesis tools for multi-step analysis and/or synthesis.

When working with fluids in conventional macroscopic volumes, fluid metering is relatively straightforward. In microfluidic volumes, however, fluid metering is considerably more difficult. Most, if not all, microfluidic systems require some interface to the conventional macrofluidic world. Using conventional macrofluidic

techniques, the smallest volume of liquid that can be generated is a droplet, typically ranging in volume between approximately one to one hundred microliters. At the low end of this volumetric range it is extremely difficult to consistently create droplets having a reasonably low volumetric standard deviation. Applications in which fluidic metering accuracy is important include microfluidic synthesis, where it would be desirable to measure stoichiometric microfluidic volumes of reagents and solvents.

It is further difficult to segregate a small fluid volume from a larger bulk volume within a microfluidic device. Such segregation requires the forces of cohesion (interaction between like fluid molecules) and adhesion (interaction between fluid molecules and the surrounding conduit) to be overcome. It is believed that the general dominance of surface effects over momentum effects in microfluidic systems contributes to the challenge of performing fluid metering within such systems.

In certain microfluidic systems it would be desirable to segregate a discrete aliquot of liquid from a substantially continuous flow of liquid. For example, in a multi-dimensional analytical system, it may be desirable to perform a first analytical technique (such as liquid chromatographic separation) on a sample, and then subject only certain portions of the eluent to further analysis (such as a mass spectrometric analysis). It would be desirable to remove one or more portions of interest from a stream of liquid, particularly if such segregation could be accomplished without introducing air into the system. Additionally, when used in an analytical system such as used for high performance liquid chromatography, it would be desirable to provide a structure capable of withstanding high pressures and withstanding chemical attack (e.g., from organic solvents) without degrading the device itself or otherwise interacting with the solvent or analyte (e.g., by acting to absorb substances from the or discharge or analyte or leaching substances into the analyte)

It may also be desirable to analyze or examine a small fluid volume while it remains contained in the microfluidic device. However, it is difficult to position a discrete microfluidic volume in a specific location within a microfluidic device (such as under an optical window) to allow such analysis to take place. The small volume of liquid, small dimensions of a microfluidic structure, and physical limitations of mechanisms for moving fluids within a microfluidic device all contribute to the difficulty in precisely positioning fluid volume within a microfluidic device.

One known method for generating small droplets is to combine fluids to be metered with surfactants before dispensing the liquid through a pipet tip. This method

is unacceptable for many applications, however, since the presence of surfactants may detrimentally compromise the purity of the fluid to be metered, and it may be very challenging to remove the surfactants and purify the fluid for further processing or use.

One method for metering small volumes of fluids is described in U.S. Patent 6,481,453, issued June 27, 2002 and commonly assigned to the owner of the present application. A primary or "trunk" channel is provided in conjunction with a vented microfluidic branch channel of a known volume. First, a fluid is directed into the trunk channel. Subsequently, a portion of the fluid is directed into the branch channel. The trunk channel may then be flushed, typically with a gas, leaving the portion of fluid in the branch channel. Because the branch channel is of a known volume, the volume of fluid contained in the branch also is known.

For trunk/branch metering system to function, however, the branch channel must be vented in some manner. If a branch channel is not vented, then any gas trapped in the branch channel by the fluid may form a bubble or otherwise occupy volume in the branch channel, thus creating error in the metered volume. The branch channel may be vented in a number of ways, including through a gas-permeable membrane that allows gas to pass through but restricts fluid flow. Also, multiple channels may be connected to a common vent channel. Alternatively, a branch channel may include a fluidic impedance that, at a given fluid pressure, prevents the flow of a liquid through the end of the branch channel while allowing gas to pass. Once the desired amount of fluid has been metered, the fluid pressure may be increased to overcome the impedance and expel the liquid volume through the impedance into a receptacle or other desirable structure or device.

Use of a permeable membrane to vent a branch channel may be undesirable because fluid may inadvertently escape through the membrane. For example, small amounts of fluid may evaporate or seep through the membrane, or small tears or holes in the membrane (which may be difficult to detect) can allow fluid to pass through the membrane. In either event, even small amounts of seepage or leakage can render the device inaccurate or inoperable. Moreover, even absent any significant leakage or seepage, the fluid may wet the porous membrane. In certain applications, devices with wetted membranes may not be re-used, as the wetting of a membrane may affect its performance in subsequent operations. Also, any fluid retained by the wetted membrane may contaminate subsequent operations.

Use of impedances to retain metered samples within a branch channel prior to dispensing also may be undesirable because fluids may be inadvertently dispensed before the metering operation is complete. For example, inadvertent over-pressurization of a branch channel may cause fluid contained therein to escape prematurely, resulting in inaccurate metering. Furthermore, if the metering operation is performed merely to sequester a given volume of the fluid for analysis and not for dispensing, then the ability to pass the metered sample to a receptacle or other structure may not be necessary.

The use of porous membranes or impedance regions may increase the difficulty of controlling the position of a discrete fluid aliquot for analysis. Because there is no mechanism for physically holding the sample aliquot in one position, such a device may require sensors and control systems to identify the location of the sample aliquot, move the aliquot to the desired location, and hold the aliquot in place during the analytical operation. The small volume of the aliquot and the small dimensions of a microfluidic structure would require very sensitive sensors and/or control systems to overcome potential error inducing factors such as hysteresis, capillary action, and other system variables.

Accordingly, there exists a need for microfluidic devices and methods capable of sequestering and/or dispensing microfluidic volumes of fluid from a larger fluid volume while minimizing the risk of premature or inadvertent release of the sample volume from the device. There also exists a need for metering devices and methods capable of sequestering microfluidic sample volumes of fluid from a larger fluid volume and holding the sample volume in a desired location for analysis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a perspective view of a portion of a closed-end metering microfluidic device according to one embodiment of the present invention, the device having a single metering channel. FIG. 1B is a top view of the portion of the device of FIG. 1A.

FIG. 2A is an exploded perspective view of a six-layer microfluidic device according to another embodiment of the present invention, the device having several parallel metering channels each capable of metering two aliquots from a larger fluidic volume. FIG. 2B is a top view of the assembled device of FIG. 2A.

FIG. 3A is a partial cross-sectional view of the device of FIGS. 2A-2B taken along section line "A"-"A" with the device in a first operational state. FIG. 3B shows

the same view as FIG. 3A, but with the device in a second operational state. FIG. 3C shows the same view as FIGS. 3A-3B, but with the device in a third operational state.

FIG. 4A is an exploded perspective view of a seven-layer microfluidic device according to another embodiment of the present invention, the device having five metering channels each capable of metering and mixing aliquots of two different fluids. FIG. 4B is a top view of the assembled device of FIG. 4A.

FIG. 5A is an exploded perspective view of a five-layer microfluidic device according to another embodiment of the present invention, the device having five metering channels and being capable of metering and mixing aliqouts of two different fluids in five different ratios. FIG. 5B is a top view of the assembled device of FIG. 5A.

FIG. 6A is an exploded perspective view of a five-layer microfluidic device according to another embodiment of the present invention, the device having five metering channels each having a different type of fluid mixing region. FIG. 6B is a top view of the assembled device of FIG. 6A.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Definitions

The term "adhesiveless" as used herein refers to the state of lacking any substance adapted to stick, bond, or otherwise adhere one surface to another.

The terms "aliquot" or "plug" as used herein refers to a discrete portion of fluid typically separated from a larger volume.

The terms "channel" or "chamber" as used herein are to be interpreted in a broad sense. Thus, they are not intended to be restricted to elongated configurations where the transverse or longitudinal dimension greatly exceeds the diameter or cross-sectional dimension. Rather, such terms are meant to comprise cavities or tunnels of any desired shape or configuration through which liquids may be directed. Such a fluid cavity may, for example, comprise a flow-through cell where fluid is to be continually passed or, alternatively, a chamber for holding a specified, discrete ratio of fluid for a specified ratio of time. "Channels" and "chambers" may be filled or may contain internal structures comprising, for example, valves, filters, and similar or equivalent components and materials.

The term "closed end" as used herein and applied to a fluidic channel refers to channel terminus through which fluid flow is not permitted.

The term "interpenetrably bound" as used herein refers to the condition of two adjacent polymer surfaces being bound along a substantially indistinct interface resulting from diffusion of polymer chains from each surface into the other.

The term "microfluidic" as used herein is to be understood to refer to structures or devices through which a fluid is capable of being passed or directed, wherein one or more of the dimensions is less than about five hundred microns, or to fluidic volumes of less than or equal to about two microliters.

The term "microfluidic impedance" as used herein is to be understood, without any restriction thereto, to refer to structures within the microfluidic device that hinder fluid flow. The shape, geometry and material that comprise these devices are not limited to the specific examples provided herein.

The term "open end" as used herein and applied to a fluidic channel refers to channel terminus through which fluid can flow.

The term "self-adhesive tape" as used herein refers to a material layer or film having an integral adhesive coating on one or both sides.

The term "substantially metal-free" as used herein means substantially free of metals, metal ions, and organometallic compounds.

The term "substantially sealed" as used herein refers to the condition of having a sufficiently low unintended leakage rate and/or leakage volume under given flow, fluid identity, or pressure conditions. Types of unintended leakage include leakage or pooling that accumulates in unintended regions between device layers and leakage to an environment outside a microfluidic device. A substantially sealed microstructure is contemplated to have one or more fluidic ports or apertures to provide desirable fluidic inlet or outlet utility.

The terms "stencil" or "stencil layer" as used herein refers to a material layer or sheet that is preferably substantially planar, through which one or more variously shaped and oriented channels have been cut or otherwise removed through the entire thickness of the layer, thus permitting substantial fluid movement within the layer (as opposed to simple through-holes for transmitting fluid through one layer to another layer). The outlines of the cut or otherwise removed portions form the lateral boundaries of microstructures that are completed when a stencil is sandwiched between other layers, such as substrates and/or other stencils. Stencil layers can be either substantially rigid or flexible (thus permitting one or more layers to be manipulated so as not to lie in a plane).

Microfluidic devices generally

In an especially preferred embodiment, microfluidic devices according to the present invention are constructed using stencil layers or sheets to define channels and/or chambers. As noted previously, a stencil layer is preferably substantially planar and has a channel or chamber cut through the entire thickness of the layer to permit substantial fluid movement within that layer. Various means may be used to define such channels or chambers in stencil layers. For example, a computer-controlled plotter modified to accept a cutting blade may be used to cut various patterns through a material layer. Such a blade may be used either to cut sections to be detached and removed from the stencil layer, or to fashion slits that separate regions in the stencil layer without removing any material. Alternatively, a computer-controlled laser cutter may be used to cut portions through a material layer. While laser cutting may be used to yield precisely dimensioned microstructures, the use of a laser to cut a stencil layer inherently involves the removal of some material. Further examples of methods that may be employed to form stencil layers include conventional stamping or die-cutting technologies, including rotary cutters and other high throughput auto-aligning equipment (sometimes referred to as converters). The above-mentioned methods for cutting through a stencil layer or sheet permits robust devices to be fabricated quickly and inexpensively compared to conventional surface micromachining or material deposition techniques that are conventionally employed to produce microfluidic devices.

After a portion of a stencil layer is cut or removed, the outlines of the cut or otherwise removed portions form the lateral boundaries of microstructures that are completed upon sandwiching a stencil between substrates and/or other stencils. The thickness or height of the microstructures such as channels or chambers can be varied by altering the thickness of the stencil layer, or by using multiple substantially identical stencil layers stacked on top of one another. When assembled in a microfluidic device, the top and bottom surfaces of stencil layers are intended to mate with one or more adjacent layers (such as stencil layers or substrate layers) to form a substantially enclosed device, typically having at least one inlet port and at least one outlet port.

A wide variety of materials may be used to fabricate microfluidic devices having sandwiched stencil layers, including polymeric, metallic, and/or composite

materials, to name a few. Various preferred embodiments utilize porous materials including filter materials. Substrates and stencils may be substantially rigid or flexible. Selection of particular materials for a desired application depends on numerous factors including: the types, concentrations, and residence times of substances (e.g., solvents, reactants, and products) present in regions of a device; temperature; pressure; pH; presence or absence of gases; and optical properties.

Various means may be used to seal or bond layers of a device together. For example, adhesives may be used. In one embodiment, one or more layers of a device may be fabricated from single- or double-sided adhesive tape, although other methods of adhering stencil layers may be used. Portions of the tape (of the desired shape and dimensions) can be cut and removed to form channels, chambers, and/or apertures. A tape stencil can then be placed on a supporting substrate with an appropriate cover layer, between layers of tape, or between layers of other materials. In one embodiment, stencil layers can be stacked on each other. In this embodiment, the thickness or height of the channels within a particular stencil layer can be varied by varying the thickness of the stencil layer (e.g., the tape carrier and the adhesive material thereon) or by using multiple substantially identical stencil layers stacked on top of one another. Various types of tape may be used with such an embodiment. Suitable tape carrier materials include but are not limited to polyesters, polycarbonates, polytetrafluoroethlyenes, polypropylenes, and polyimides. Such tapes may have various methods of curing, including curing by pressure, temperature, or chemical or optical interaction. The thickness of these carrier materials and adhesives may be varied.

Device layers may be directly bonded without using adhesives to provide high bond strength (which is especially desirable for high-pressure applications) and eliminate potential compatibility problems between such adhesives and solvents and/or samples. For example, in one embodiment, multiple layers of 7.5-mil (190 micron) thickness, adhesiveless "Clear Tear Seal" polypropylene (American Profol, Cedar Rapids, IA) including at least one stencil layer may be stacked together, placed between glass platens and compressed to apply a pressure of 0.26 psi (1.79 kPa) to the layered stack, and then heated in an industrial oven for a period of approximately five hours at a temperature of 154 °C to yield a permanently bonded microstructure well-suited for use with high-pressure column packing methods. In another embodiment, multiple layers of 7.5-mil (188 micron) thickness "Clear Tear Seal"

polypropylene (American Profol, Cedar Rapids, IA) including at least one stencil layer may be stacked together. Several microfluidic device assemblies may be stacked together, with a thin foil disposed between each device. The stack may then be placed between insulating platens, heated at 152°C for about 5 hours, cooled with a forced flow of ambient air for at least about 30 minutes, heated again at 146°C for about 15 hours, and then cooled in a manner identical to the first cooling step. During each heating step, a pressure of about 0.37 psi (2.55 kPa) is applied to the microfluidic devices.

Notably, stencil-based fabrication methods enable very rapid fabrication of devices, both for prototyping and for high-volume production. Rapid prototyping is invaluable for trying and optimizing new device designs, since designs may be quickly implemented, tested, and (if necessary) modified and further tested to achieve a desired result. The ability to prototype devices quickly with stencil fabrication methods also permits many different variants of a particular design to be tested and evaluated concurrently.

Further embodiments may be fabricated from various materials using well-known techniques such as embossing, stamping, molding, and soft lithography.

In addition to the use of adhesives and the adhesiveless bonding method discussed above, other techniques may be used to attach one or more of the various layers of microfluidic devices useful with the present invention, as would be recognized by one of ordinary skill in attaching materials. For example, attachment techniques including thermal, chemical, or light-activated bonding steps; mechanical attachment (such as using clamps or screws to apply pressure to the layers); and/or other equivalent coupling methods may be used.

Preferred embodiments

Referring to FIGS. 1A-1B, a microfluidic device 10 according to a first embodiment includes three device layers 17-19. The first layer 17 defines a microfluidic actuating channel 12. The third layer 19 defines a microfluidic metering channel 14. Preferably, the device 10 further includes upper and lower boundary or cover layers (not shown) to enclose the microstructures 12, 14, 20, 22 defined in the two outermost illustrated layers 17, 19. The actuating channel 12 and metering channel 14 are physically separate, that is, fluid cannot be communicated between them. The metering channel 14 is in fluid communication with a larger volume of fluid

to be sampled (a "fluid source" 16 indicated schematically with an arrow at the proximate end of the metering channel 14) . The fluid source 16 may be any macro- or microfluidic structure, including, but not limited to a trunk channel, a reaction chamber, or a reservoir. The actuating channel 12, which can use air or liquid as an operant fluid, is separated from the metering channel 14 by an intermediate layer 18, which is preferably a deformable membrane.

Increasing or decreasing the pressure within the actuating channel 12 (the "actuating pressure") causes the deformable membrane 18 to deform accordingly, thus altering the volume of the metering channel 14. The metering channel 14 is a closed-end channel, i.e., it has only one inlet and fluid cannot flow through the closed end. The deformable membrane 18 is adapted to selectively alter the volume of the actuating channel 12, and concomitantly the volume of the metering channel 14 since the deformable membrane 18 bounds a portion of each channel 12, 14. Changes in the volume of the metering channel 14 result in a pressure change in metering channel 14, thus creating a pressure differential between the metering channel 14 and the fluid source 16 (the "metering pressure"). The metering pressure causes a fluid aliquot to be drawn into or expelled from the metering channel 14. For example, decreases in metering pressure can act to draw the fluid aliquot from the fluid source 16 into the metering channel 14. Conversely, increases in metering pressure can act to push a fluid aliquot from the metering channel 14 in the direction of the fluid source 16.

One or both of the actuating channel 12 and the metering channel 14 may have an enlarged or "control" portion 20, 22 (respectively) having at least one dimension that that is significantly different than the dimensions of the associated channel 12, 14. For example, as shown in FIGS. 1A-1B, each channel 12, 14 has an associated enlarged portion 20, 22, each being approximately circular in shape and of equal size, with both enlarged portions 20, 22 being substantially larger than the remainder of its associated channel 12, 14. At least one enlarged portion 22 is preferably disposed proximate to the closed end of the metering channel 14. It will be readily apparent to one skilled in the art that the relative geometry and size of the control portions 20, 22 may be varied to achieve desired results. Volumetric differences between the two control portions 20, 22 allow small changes in actuating pressure to have either much greater or much smaller effect on the resultant draw produced by the metering channel 14. Also, significant differences in volume

between the control portions 20, 22, allow for small variations in actuating pressure to have an amplified or attenuated effect on metering pressure. Thus, the gain of the system may be controlled.

For example, a large actuating channel control portion 20 used in combination with a relatively small metering control portion 22 will allow small changes in control pressure to effect large changes in metering pressure, thus amplifying the control signal. Likewise, a relatively small actuating channel control portion 20 used in combination with a larger metering control portion 22 will allow large changes in control pressure to be made with very small resulting changes in metering pressure, thus attenuating the control signal.

When enlarged control portions are used, and particularly when metering channel control portions are substantially larger than the dimensions of the remainder of the metering channel, care should be taken to avoid drawing fluid into the control portion. It may be difficult to accurately measure the volume of a fluid aliquot when a portion of the aliquot is drawn into the control portion. This is because the aliquot may not completely fill the control portion. Also, if and when the aliquot is expelled from the measuring channel, some of the aliquot may remain trapped in the control portion, inducing further inaccuracy, as well as potentially contaminating aliquots of other fluids subsequently drawn into the metering channel. In order to avoid these problems, the volume of the portion of the metering channel between fluid source and the control portion(s) (the "metering region") is preferably larger than the maximum change in volume that can be created by the associated control portions. In other words, the volume defined by the enlarged portion 22 associated with the metering channel 14 may be selected to prevent introduction of liquid into the enlarged portion 22 when the deformable membrane 18 is manipulated from a full inward position (such as may be caused by an elevated pressure within in the actuating channel 12 relative to the pressure within the metering channel 14) to a full outward position (such as may be caused by a reduction of pressure in the actuating channel 12 relative to the pressure within the metering channel 14).

Microfluidic closed-end metering devices according to the invention may be incorporated into more complex structures. For example, a single actuating channel may actuate multiple metering channels. Likewise, a metering channel may be actuated by multiple actuating channels. Any number of metering channels and

actuating channels, in any combination or geometry may be used to perform the desired metering and/or sequestering operations.

In addition, at least a portion of the metering region may be fabricated with a substantially optically transmissive material to permit analysis of the fluid aliquot while it is sequestered in the metering region. For example, the device layers may be a substantially optically transmissive polymer such as polypropylene or other suitable polymers. Alternatively, a window fabricated with quartz, glass or any other suitable material may be included in one or more of the device layers at the desired location.

Referring to FIGS. 2A-2B, a microfluidic device 100 includes six metering channels 110A-110N, each with two control portions 113A-113N, 114A-114N, and four separate actuating channels 116-119, each having three control portions 116A-116N, 117A-117N, 118A-118N, 119A-119N. (Although FIGS. 2A-2B show the device 100 as having six metering channels 110A-110N and four actuating channels 116A-116N, it will be readily apparent to one skilled in the art that any number of metering and actuating channels may be provided. For this reason, the designation "N" is used to represent the last metering channel 110N and last control portions 116N-119N with the understanding that "N" represents a variable and could represent any desired number of such channels or any other feature or structure within the device.)

The microfluidic device 100 is constructed with six device layers 121-126. The first device layer 121 is a 0.0625 inch (1.6 mm) thick acrylic substrate. The first device layer 121 defines trunk channel input/output ports ("I/O ports") 102, 103 and actuating channel I/O ports 106-109.

The second device layer 122 comprises a double-sided self-adhesive tape material made of a one-mil (25 micron) thick polypropylene carrier with a two mil (50 micron) thickness coating of rubber adhesive on both side. The second device layer 122 is a stencil layer that defines a trunk channel 132 and four separate actuating channels 116-119, each having three control portions 116A-116N, 117A-117N, 118A-118N, 119A-119N, respectively. Each actuating channel 116-119 is in fluid communication with one of the actuating channel I/O ports 106-109. The trunk channel 132 is in fluid communication with the trunk channel I/O ports 102, 103.

The third device layer 123 is a one-half mil (12 micron) thick polypropylene film that defines metering channel vias 134A-134N. (A "via" is an aperture providing fluid communication between non-adjacent device layers.) At least the portion of the third device layer 123 between the actuating channel control portions 116A-116N, 117A-

117N, 118A-118N, 119A-119N and the measuring channel control portions 113A-113N, 114A-114N is a deformable membrane, and preferably an elastically deformable membrane (i.e., such that following application of a force that deforms the membrane 123 along one or more control portions 116A-116N, 117A-117N, 118A-118N, 119A-119N, the device layer 123 will return substantially to its pre-stressed state under the typical operating conditions of the device 100). In the embodiment shown in FIGS. 2A-2B, the entire third device layer 123 is fabricated with a material that functions as a deformable membrane; however, a deformable membrane region (not shown) could be inset into an otherwise non-deformable third device layer 123 in the area between the actuating channel control portions 116A-116N, 117A-117N, 118A-118N, 119A-119N and the metering channel control portions 113A-113N, 114A-114N.

The fourth device layer 124 comprises a double-sided self-adhesive tape material made of a one-mil (25 micron) thick polypropylene carrier with a two mil (50 micron) thickness coating of rubber adhesive on both sides. The fourth device layer 124 is a stencil layer that defines six metering channels 110A-110N, each having an open end 111A-111N and a closed end 112A-112N. Each metering channel 110A-110N includes two control portions 113A-113N, 114A-114N. The metering channels 110A-110N are in fluid communication with the trunk channel 132 through metering channel vias 134A-134N.

The fifth and sixth device layers 125, 126 serve to cover the microstructures defined in the fourth layer 124. The fifth and sixth device layers 125, 126 are fabricated with two-mil (50 micron) thickness polypropylene film.

Referring to FIGS. 2A-2B and 3A-3C, in operation of the device 100, a pressure is applied to actuating channel I/O ports 106-109 to initially pressurize actuating channels 116-119 to approximately 10 psi (69 kPa). Note that FIG. 3A shows the device 100 in a passive state, with neither pressure nor vacuum applied to the actuating channels 116-119. A fluid to be sampled is provided to the trunk channel 132 through either of the trunk channel I/O ports 102, 103. Referring to FIG. 3B, vacuum (i.e., sub-atmospheric pressure such as may be provided by an vacuum pump (not shown) external to the device 100) is then applied to a first actuating channel 118, including actuating channel control portions 118A-118N. The portion of the flexible membrane device layer 122 between the actuating channel control portions 118A-118N and the metering control portions 113A-113C is drawn downward

into the actuating channel control portions 118A-118N. This deformation of the flexible membrane device layer 122 expands the volume of the metering channel control portions 113A-113C. Because the metering channels 110A-110C are "closed-end" channels (i.e., open only to the trunk channel 132), the increased volume creates a pressure differential between the metering channels 110A-110C and the trunk channel 132. This pressure differential causes a first fluid aliquot 150 to be drawn from the trunk channel 132 into each of the metering channels 110A-110C, as shown in FIG. 3B. Subsequently or simultaneously, a vacuum may be applied to the adjacent actuating channel 116, thereby creating additional vacuum to draw a second fluid aliquot 152 into the metering channels 110A-110C, as shown in FIG. 3C. When the actuating channels 116, 118 are re-pressurized, the fluid aliquots 150, 152 within each of the metering channels 110A-110C are pushed back into the trunk channel 132. This can be accomplished in one step by re-pressurizing both the actuating channels 116, 118 simultaneously, or in steps, by re-pressurizing each actuation channel 118, 116 in sequence.

It will be readily apparent to one skilled in the art that any suitable mechanism for deforming the deformable membrane 122 may be employed. While a pressure source (such as a reservoir of pressurized gas) and vacuum source (such as a vacuum pump) may be used, alternative embodiments may include magnetic, mechanical, or electromechanical actuators. For example, a magnetic element may be incorporated in or affixed to the deformable membrane 122 and a magnetic field applied to move the membrane 122 upward or downward. Similarly, a piezoelectric element may be incorporated in the device 100, such as by being affixed to the membrane 122. Various pumps may also be used, including reversible pumps such as conventional syringe pumps.

Closed-ended microstructures may also be used to mix discrete fluid volumes. For example, FIGS. 4A-4B illustrate a microfluidic device 200 comprising seven device layers 201-207 and defining five metering channels 250A-250N each permitting aliquots of two different fluids to be metered and mixed. The first through third layers 201-203 and fifth through seventh layers 205-207 are made of 7.5 mil (190 micron) thickness polypropylene film. The fourth layer 204 is made of 2 mil (50 micron) thickness polypropylene film. All of the device layers 201-207 are adhesiveless and substantially metal-free. The layers 201-207 are interpenetrably bonded together according to an adhesiveless bonding procedure discussed

previously herein to form a substantially sealed microstructure. Notably, when such an adhesiveless bonding procedure is used, the resulting device 200 is capable of operation at higher pressures than might be used with a similar device bonded using conventional adhesives. Applicants have routinely operated similar adhesiveless devices with internal pressures greater than 500 psi (3450 kPa). However, Applicants have found that for purposes of pressure-based actuation of closed-ended devices as described herein, actuating pressures of up to 10-20 psi (69-138 kPa) are typically sufficient to operate the devices as intended. Lower or higher actuating pressures may be used, as would be recognized by one skilled in the art.

The first layer 201 defines three trunk channel I/O ports 210-212. Preferably, different fluids are supplied through the first two ports 210, 211, and the fluid flows through the trunk channel 222 (defined in the second layer 202) in the direction of the third port 212. The first layer 201 further defines three actuating channel I/O ports 214-216, each permitting fluid communication with a different actuating channel 224-226, respectively, defined in the second layer 202.

The second layer 202 is a stencil layer defining various channel structures. The trunk channel 222 includes two inlet segments 220, 221. Three actuating channels 224-226 each define five control portions 224A-224N, 225A-225N, 226A-226N, respectively.

The third layer 203 defines five metering channel vias 231A-231N in fluid communication with the trunk channel 222, and defines three sets of five actuating channel vias 234A-234N, 235A-235N, 236A-236N each in fluid communication with a different control portion 224A-224N, 225A-225N, 226A-226N defined in the second layer 202.

The fourth layer 204, which serves as a deformable membrane, defines five metering channel vias 232A-232N aligned with corresponding vias 231A-231N defined in the third layer 203.

The fifth layer 205 is substantially identical to the third layer 203; it defines five metering channel vias 233A-233N aligned with the vias 231A-231N and 232A-232N defined in the third and fourth layers 203, 204, respectively. The fifth layer 205 also defines three sets of five metering channel vias 244A-244N, 245A-245N, 246A-246N.

The sixth layer 206, which is a stencil layer, defines five metering channels 250A-250N each having an open end 251A-251N and a closed end 252A-252N. Each metering channel 250A-250N includes an upstream portion 257A-257N.

Although the upstream portions 257A-257N are shown as having a serpentine shape to provide increased channel length within a compact space, the portions 257A-257N may be straight or configured in any suitable shape. Each metering channel 250A-250N further includes three metering channel control portions 254A-254N, 255A-255N, 256A-256N. Each control portion 254A-254N, 255A-255N, 256A-256N is aligned with one corresponding metering channel via 244A-244N, 245A-245N, 246A-246N defined in the sixth layer 206. The seventh layer 207 serves as a cover for various microstructures defined in the layers below.

In operation of the device 200, a pressure is applied to the actuating channel I/O ports 214-216 to initially pressurize the actuating channels. As noted previously, pressures between 10-20 psi (69-138 kPa) may be used, although lower or higher actuating pressures may also be used. A first fluid to be sampled is provided to the trunk channel 222 by way of a first trunk channel I/O port 210 and channel segment 220. Vacuum may then be applied to a first actuating channel 224, including actuating channel control portions 224A-224N. The portions of the flexible membrane device layer 204 between the actuating channel control portions 224A-224N and the corresponding metering control portions 254A-254N are drawn downward into at least the actuating channel vias 234A-234N and preferably farther into the actuating channel control portions 224A-224N. This serves to increase the volume of each of the metering channels 250A-250N, and because the metering channels each have a closed end 252A-252N, the increased volume creates a first pressure differential between the metering channels 250A-250N and the trunk channel 222. This pressure differential causes a first fluid aliquot (not shown) to be drawn into each metering channel 250A-250N.

Subsequently, a second fluid (not shown) is provided to the trunk channel 222 (preferably in lieu of the first fluid) by way of a second trunk channel I/O port 211 and corresponding channel segment 221. Vacuum is then applied to the second actuating channel 225, including actuating channel control portions 225A-225N. The portions of the flexible membrane device layer 204 between the actuating channel control portions 225A-225N and the corresponding metering control portions 255A-255N are drawn downward. This serves to further increase the volume of each of the metering channels 250A-250N, and to create a second pressure differential between the metering channels 250A-250N and the trunk channel 222 that causes a second

fluid aliquot (not shown) to be drawn into each metering channel 250A-250N behind each first fluid aliquot (not shown).

While two static microfluidic aliquots placed into contact with one another will eventually mix through diffusion, such mixing is generally proportional to the diffusion interface area and the average diffusional path length between the two aliquots. In this case, the diffusional interface area between the first fluid aliquot and the second fluid aliquot in the metering channels 250A-250N is quite small, and the average diffusional path length between the aliquots is large. As a result, static diffusional mixing will take an exceptionally long time. Mixing of the first fluid aliquot and second fluid aliquot may be accelerated by moving the two aliquots within each metering channel 250A-250N by deforming the membrane 204 adjacent to the actuation control regions 226A-226N of the third actuating channel 226. Specifically, elevated pressure and vacuum may be alternatively applied to the third actuating channel 226 to cause the two fluid aliquots to move back and forth within the upstream portions 257A-257N of the metering channels 250A-250N. While the precise mixing mechanism is not fully understood, it is believed that as the aliquots flow through each metering channel 250A-250N, molecules of a first fluid temporarily "stick" to the channel walls and intermingle with molecules of a second fluid as they flow past. Generally, complete mixing can be effected after repeated cycles of back-and-forth motion within the metering channels 250A-250N. Following mixing, the mixed fluids may be retained within the metering channels 250A-250N (such as may be useful for analysis of the fluids, e.g., by optical detection methods) or the actuating channels 254-256 may be pressurized to expel the mixed fluids back into the trunk channel 222 for subsequent use or analysis.

In another embodiment, a microfluidic device having closed-ended channels may be used to meter and mix aliquots of different fluids in multiple different mixing ratios. For example, FIGS. 5A-5B illustrate a microfluidic device 300 comprising five device layers 301-305 and defining five metering channels 350A-350N each permitting aliquots of different fluids to be metered and mixed in different ratios. The first device layer 301 is made of a 0.0625 inch (1.6 mm) thickness acrylic substrate. The second and fourth device layers 302, 304 are each made of five mil (125 microns) thickness double-sided self-adhesive tape comprising a one mil (25 microns) thickness polyester carrier with a two mil (50 microns) thickness coating of rubber adhesive on both sides. The third device layer 303 is made with a two mil (50

microns) thickness polypropylene film. The fifth layer 305 is made with a 7.5 mil (190 microns) thickness polypropylene film. As shown in FIG. 5B, the resulting 300 device is similar in appearance to the device 200 of the previous embodiment.

The first layer 301 defines three trunk channel I/O ports 310-312. Preferably, different fluids are supplied through the first two ports 310, 311, and the fluid flows through the trunk channel 322 (defined in the second layer 302) in the direction of the third port 312. The first layer 301 further defines three actuating channel I/O ports 314-316, each permitting fluid communication with a different actuating channel 324-326, respectively, defined in the second layer 302.

The second layer 302 is a stencil layer defining a trunk channel 322 having two inlet segments 320, 321. The second layer 302 further defines three actuating channels 324-326 that each include five control portions 324A-324N, 325A-325N, 326A-326N, respectively. Notably, each of the first actuating channel control portions 324A-324N is of slightly different size, with the smallest control portion being closest to the first actuating channel port 314 ascending in size to the largest control portion 324N disposed at the distal end of the first actuating channel 324. Further, each of the second actuating channel control portions 325A-325N is of a slightly different size, but order of the sizes is reversed relative to the first actuating channel. Namely, the largest control portion 325A is closest to the second actuating channel port 315, and further control portions 325B-325D ascend in size to the largest control portion 325N, which is disposed at the distal end of the second actuating channel 325. Each of the third actuating channel control portions 326A-326N is of the same size.

The third layer 303, which serves as a deformable membrane, defines five metering channel vias 332A-332N each in fluid communication with the trunk channel 322 defined in the second layer 302.

The fourth layer 304, which is a stencil layer, defines five metering channels 350A-350N each having an open end 351A-351N and a closed end 352A-352N. Each metering channel 350A-350N includes an upstream portion 357A-357N, each here configured in a serpentine shape to provide increased channel length within a compact space. Each metering channel 350A-350N further includes three metering channel control portions 354A-354N, 355A-355N, 356A-356N. Each metering channel control portion 354A-354N, 355A-355N, 356A-356N is aligned with, and sized and shaped according to, the corresponding actuating channel control portions

324A-324N, 325A-325N, 326A-326N defined in the second layer 302. The fifth layer 305 serves as a cover for various microstructures defined in the layers below.

In operation of the device 300, a pressure is applied to the actuating channel I/O ports 314-316. A first fluid to be sampled is provided to the trunk channel 322 by way of a first trunk channel I/O port 310 and channel segment 320. Vacuum may then be applied to a first actuating channel 324, including actuating channel control portions 324A-324N. The portions of the flexible membrane device layer 303 between the actuating channel control portions 324A-324N and the corresponding metering control portions 354A-354N are drawn downward, to create a first pressure differential between the metering channels 350A-350N and the trunk channel 322. This pressure differential causes a first fluid aliquot (not shown) to be drawn into each metering channel 350A-350N. Since the volume of each aliquot varies in proportion to the change in volume of each metering channel first control portion 324A-324N, and each metering channel first control portion 324A-324N, aliquots of different volumes are drawn into each metering channel 350A-350N.

Subsequently, a second fluid (not shown) is provided to the trunk channel 322 (preferably in lieu of the first fluid) by way of a second trunk channel I/O port 311 and corresponding channel segment 321. Vacuum is then applied to the second actuating channel 325, including actuating channel control portions 325A-325N. The portions of the flexible membrane device layer 303 between the actuating channel control portions 325A-225N and the corresponding metering control portions 355A-355N are drawn downward. This serves to further increase the volume of each of the metering channels 350A-350N, and to create a second pressure differential between the metering channels 350A-350N and the trunk channel 322 that causes a second fluid aliquot (not shown) to be drawn into each metering channel 350A-350N behind each first fluid aliquot (not shown). For the same reason noted above, the volume of each second aliquot varies, such that aliquots of different volumes are drawn into each metering channel 350A-350N. What results is a relatively small aliquot of a first fluid in contact with a relatively large aliquot of a second fluid in the first metering channel 350A, ranging to a relatively large aliquot of a first fluid in contact with a relatively small aliquot of a second fluid in the fifth metering channel 350N.

Mixing of the first and second aliquot in each metering channel 350A-350N proceeds as in the previous example, by deforming the deformable membrane 303 (preferably from a full inward to a full outward position) along the third actuation

channel control regions 326A-326N. This may be performed by alternately applying pressure and vacuum to the third actuation channel 326, which causes the aliquots contained in each metering channel 350A-350N to travel back and forth within the upstream portions 357A-357N. Notably, a large number of different mixtures of two or more fluids can be obtained with a relatively small number of actuation channels (here, only three actuation channels 324-326).

While the preceding two embodiments including back-and-forth mixers shorten the mixing time compared to static diffusive mixing, further embodiments including one or more contraction/expansion region may provide even more rapid fluid mixing utility. For example, FIGS. 6A-6B illustrate a microfluidic device 400 comprising five device layers 401-405 and defining five metering channels 450A-450N each demonstrating a different fluid mixer. The first device layer 401 is made of a 0.0625 inch (1.6 mm) thickness acrylic substrate. The second and fourth device layers 402, 404 are each made of five mil (125 microns) thickness double-sided self-adhesive tape comprising a one mil (25 microns) thickness polyester carrier with a two mil (50 microns) thickness coating of rubber adhesive on both sides. The third device layer 403 is made with a two mil (50 microns) thickness polypropylene film. The fifth layer 405 is made with a 7.5 mil (190 microns) thickness polypropylene film.

The first layer 401 defines three trunk channel I/O ports 410-412, with the preferable direction of fluid flow being from the first two ports 410, 411, toward the third port 412. The first layer 301 further defines three actuating channel I/O ports 414-416, each permitting fluid communication with a different actuating channel 424-426, respectively, defined in the second layer 402.

The second layer 402 is a stencil layer defining a trunk channel 422 having two inlet segments 420, 421. The second layer 402 further defines three actuating channels 424-426 that each include five control portions 424A-424N, 425A-425N, 426A-426N, respectively. The second layer 402 also defines two oval-shaped channel segments 470A, 470C that serve as a portion of one composite metering channel 450A originating in the fourth layer 404.

The third layer 403, which serves as a deformable membrane, defines five metering channel vias 432A-432N each in fluid communication with the trunk channel 422 defined in the second layer 402. The third layer 403 further defines four narrow channel segments 460A-460D that serve as further portions of one composite metering channel 450A originating in the fourth layer 404.

The fourth layer 404, which is a stencil layer, defines (at least portions of) five metering channels 450A-450N. Each metering channel 450A-450N has an open end 451A-451N and a closed end 452A-452N. Each of the first four metering channels 450A-450D define contraction/expansion regions of differing configurations. The first metering channel 450A is a composite channel defined in three device layers 402-404; it includes narrow segments 460A-460D that connect oval-shaped segments 470A-470C defined in the third and fourth layers 403, 404. Fluid flow through these interconnected segments contracts each time it enters a narrow segment 460A-460D and expands each time it enters an oval-shape segment 470A-470D. In the aggregate, the narrow segments 460A-460D and oval-shaped segments 470A-470D will herein be referred to as a first contraction/expansion region 481.

The second through fourth metering channels 450B-450D are defined exclusively in the fourth layer 404. The second metering channel 450B includes an oval-shaped region 461 that presents an expanding and contracting fluid flow area, and may also be referred to as a second contraction/expansion region 461. The third metering channel 450C defines four circular portions 462A-462D that also present expanding and contracting fluid flow areas for fluids flowing therethrough. The fourth metering channel defines two oval-shaped regions 463A-463B disposed in series; these also present expanding and contracting fluid flow areas to enhance fluidic mixing. The fifth layer 405 serves as a cover to enclose various microstructures defined in the layers below.

The device 400 is operated similarly to the preceding two embodiments. The pressure within the first and second actuating channels 424, 425 may be manipulated to draw first and second plugs into the first through fifth metering channels 451A-451N. Thereafter, elevated pressure and vacuum may be alternatively applied to the third actuating channel 426 to cause the two fluid aliquots in each metering channel 450A-450N to move back and forth. In the case of the first metering channel 450A, the aliquots move back and forth through the contraction/expansion region 481 comprising the narrow segments 460A-460D and oval-shaped segments 470A-470D. In the second metering channel 450B, the aliquots move back and forth through the oval-shaped region 461. In the third metering channel 450C, the aliquots move back and forth through the four circular portions 462A-462D. In the fourth metering channel 450D, the aliquots move back and forth through the two oval shaped regions 463A, 463B. Each of the preceding four metering channels 450A-450D included a

contraction/expansion region. The fifth metering channel 450N lacks such a region; instead it includes a serpentine-shaped upstream portion 450N. Notably, under most flow conditions, more rapid mixing is observed within any of the first four metering channels 450A-450D having contraction/expansion regions than in the fifth metering channel 450N that lacks such a region.

In any of the preceding embodiments, it also will be readily apparent to one skilled in the art that multiple deformable membranes may be used. For example, actuating channels may be provided above and below the metering channel, requiring at least one membrane between each actuating channel and the metering channel. Also, a single device layer may be fabricated with multiple deformable membrane segments where each segment is associated with one or more control portions and has a characteristic modulus of elasticity tailored to exhibit desired performance characteristics.

Various microfluidic device embodiments disclosed herein allow accurately measured microfluidic volumes of fluid to be withdrawn (sampled) from and returned to a larger fluidic volume. Various embodiments may also serve as a means of storing multiple small samples that have been measured by another process and that need to be sequestered from the fluid source. Samples may be moved within a defined, closed area (e.g., for mixing of discrete fluid volumes). Samples also may be moved fixed, predetermined distances reliably and repeatedly, thus minimizing the need for complex control systems that may require timers and/or sensors.

Because closed-end microfluidic metering device embodiments as described herein rely on a pressure differential between a fluid source and a metering channel to move fluid samples, the need for vents and/or gas-permeable membranes is eliminated. Thus, there is no likelihood of fluids from escaping through a vent or the system becoming inoperable or contaminated by a wetted gas-permeable membrane.

It is also to be appreciated that the foregoing description of the invention has been presented for purposes of illustration and explanation and is not intended to limit the invention to the precise manner of practice herein. It is to be appreciated therefore, that changes may be made by those skilled in the art without departing from the spirit of the invention and that the scope of the invention should be interpreted with respect to the following claims.

What is claimed is:

1. A microfluidic device (100, 200, 300, 400) comprising:

a first fluidic channel (132, 222, 322, 422) having a fluidic inlet (102, 210, 211, 310, 311, 410, 411) and a fluidic outlet (103, 212, 312, 412);

a second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) having an open end (111A-111N, 251A-251N, 351A-351N, 451A-451N) and a closed end (112A-112N, 252A-252N, 352A-352N, 452A-452N), the open end (111A-111N, 251A-251N, 351A-351N, 451A-451N) being in fluid communication with the first fluidic channel (132, 222, 322, 422);

a first deformable membrane (123, 204, 303, 403) bounding at least a portion of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N); and

means for deforming the first deformable membrane (123, 204, 303, 403);

wherein the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) is microfluidic, and the first deformable membrane (123, 204, 303, 403) is adapted to selectively alter the volume of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) to create a first pressure differential between the first fluidic channel (132, 222, 322, 422) and the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N).

2. The device of claim 1 wherein the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) includes an enlarged portion (114A-114N, 256A-256N, 356A-356N, 456A-456N) proximate to the closed end (112A-112N, 252A-252N, 352A-352N, 452A-452N), and a surface of the enlarged portion (114A-114N, 256A-256N, 356A-356N, 456A-456N) is bounded by the first deformable membrane (123, 204, 303, 403).

3. The device of claim 2 wherein:

the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) includes a first portion (257A-257N, 357A-357N, 481, 461, 462A-462D, 463A-463B, 457N) defining a first volume and the enlarged portion (114A-114N, 256A-256N, 356A-356N, 456A-456N) defines a second volume;

the first deformable membrane (123, 204, 303, 403) is capable of being manipulated into a full inward position and into a full outward position; and

the first volume and the second volume are selected such that when a liquid is introduced into the first fluidic channel (132, 222, 322, 422) and the first deformable membrane (123, 204, 303, 403) is manipulated from the full inward position to the full outward position, introduction of liquid into the enlarged portion (114A-114N, 256A-256N, 356A-356N, 456A-456N) is prevented.

4. The device of claim 1, further comprising a first actuating channel (116-119, 224-226, 324-326, 424-426), at least a portion of the first actuating channel (116-119, 224-226, 324-326, 424-426) being bounded by the first deformable membrane (123, 204, 303, 403).

5. The device of claim 1, further comprising a second deformable membrane (123, 204, 303, 403) bounding at least a portion of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N), wherein the second deformable membrane (123, 204, 303, 403) is adapted to selectively alter the volume of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) to create a second pressure differential between the first fluidic channel (132, 222, 322, 422) and the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N).

6. The device of claim 5, further comprising a second actuating channel (116-119, 224-226, 324-326, 424-426), at least a portion of the second actuating channel (116-119, 224-226, 324-326, 424-426) being bounded by the first deformable membrane (123, 204, 303, 403).

7. The device of claim 5, further comprising a third deformable membrane (123, 204, 303, 403) bounding at least a portion of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N), wherein the third deformable membrane (123, 204, 303, 403) is adapted to selectively alter the volume of the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) to create a third pressure differential between the first fluidic channel (132, 222, 322, 422) and the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N).

8. The device of claims 5-7, further comprising at least one contraction / expansion region (461, 462A-462D, 463A-463B, 481) in fluid communication within the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N).

9. The device of claims 5 or 6 wherein the first deformable membrane (123, 204, 303, 403) and the second deformable membrane (123, 204, 303, 403) are substantially continuous.

10. The device of claims 1-3, further comprising a comprising a first magnetic element associated with the first deformable membrane (123, 204, 303, 403).

11. The device of any of the preceding claims, wherein the second fluidic channel (110A-110N, 250A-250N, 350A-350N, 450A-450N) includes a detection region.

12. The device of claim 11 wherein the detection region is substantially optically transmissive.

13. The device of any of the preceding claims wherein the device comprises a plurality of device layers (121-126, 201-207, 301-305, 401-405).

14. The device of claim 13 wherein any device layer (121-126, 201-207, 301-305, 401-405) of the plurality of device layers (121-126, 201-207, 301-305, 401-405) comprises a self-adhesive tape material.

15. The device of claims 13 or 14 wherein at least one device layer (121-126, 201-207, 301-305, 401-405) of the plurality of device layers (121-126, 201-207, 301-305, 401-405) is a stencil layer (122, 124, 202, 206, 302, 304, 402, 404) defining at least one microfluidic channel.

16. The device of claims 13-15 wherein the plurality of device layers (121-126, 201-207, 301-305, 401-405) comprise a polymeric material.

17. The device of claim 16 wherein the polymeric material is substantially optically transmissive.

18. The device of claim 16 wherein the polymeric material is selected from the group consisting of: polyolefins and vinyl-based (alkene-based) polymers.

19. The device of claims 13 or 15-18 wherein the device layers (121-126, 201-207, 301-305, 401-405) are substantially metal-free, adhesiveless, and interpenetrably bound together to form a substantially sealed microstructure (100, 200, 300, 400).

20. The device of any of the preceding claims wherein the deforming means comprises any of: a pressure source, a vacuum source, at least one pump, a reversible pump, a magnetic actuator, a mechanical actuator, and an electromechanical actuator.

21. The device of any of the preceding claims wherein the first fluidic channel (132, 222, 322, 422) is microfluidic.

22. The device of any of the preceding claims wherein the first fluidic channel (132, 222, 322, 422) contains a substantially continuous flow of a liquid.

23. A method for segregating at least one microfluidic aliquot from a larger fluidic volume, the method comprising the steps of:

providing a fluid source (16, 132, 222, 322, 422);

providing a microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N) having an open end (111A-111N, 251A-251N, 351A-351N, 451A-451N) and a closed end (112A-112N, 252A-252N, 352A-352N, 452A-452N), the open end (111A-111N, 251A-251N, 351A-351N, 451A-451N) being in fluid communication with the fluid source (16, 132, 222, 322, 422);

providing a first deformable membrane (123, 204, 303, 403) that bounds at least a portion of the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N); and

deforming the first deformable membrane (123, 204, 303, 403) to draw a first fluid aliquot from the fluid source (16, 132, 222, 322, 422) into the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N).

24. The method of claim 23, further comprising the step of providing a first actuating channel (12, 116-119, 224-226, 324-326, 424-426), at least a portion of the first actuating channel (12, 116-119, 224-226, 324-326, 424-426) being bounded by the first deformable membrane (123, 204, 303, 403), wherein the step of deforming the first deformable membrane (123, 204, 303, 403) is performed by altering the pressure within the first actuating channel (12, 116-119, 224-226, 324-326, 424-426).

25. The method of claim 24, further comprising the steps of:

providing a second deformable membrane (123, 204, 303, 403) that bounds at least a portion of the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N); and

deforming the second deformable membrane (123, 204, 303, 403) to draw a second fluid aliquot from the fluid source into the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N).

26. The method of claim 25, further comprising the step of providing a second actuating channel (116-119, 224-226, 324-326, 424-426), at least a portion of the second actuating channel (116-119, 224-226, 324-326, 424-426) being bounded by the second deformable membrane (123, 204, 303, 403), wherein the step of deforming the second deformable membrane (123, 204, 303, 403) is performed by altering the pressure within the second actuating channel (116-119, 224-226, 324-326, 424-426).

27. The method of claim 23, further comprising the step of providing a first magnetic element associated with the first deformable membrane (123, 204, 303, 403), wherein the step of deforming the first deformable membrane (123, 204, 303, 403) is performed by applying a first magnetic field to the first magnetic element.

28. The method of claim 25, further comprising the step of providing a second magnetic element associated with second deformable membrane (123, 204, 303, 403), wherein the step of deforming the second deformable membrane (123, 204, 303, 403) is performed by applying a second magnetic field to the second magnetic element.

29. The method of claims 23-28, further comprising the step of deforming any of the first deformable membrane (123, 204, 303, 403) and the second deformable membrane (123, 204, 303, 403) to expel the microfluidic aliquot from the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N).

30. The method of claim 25, further comprising the steps of:

providing a contraction / expansion region (461, 462A-462D, 463A-463B, 481) in fluid communication with the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N);

deforming the first deformable membrane (123, 204, 303, 403) to draw a first microfluidic aliquot into the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N);

deforming the second deformable membrane (123, 204, 303, 403) to draw a second microfluidic aliquot into the microfluidic channel (14, 110A-110N, 250A-250N, 350A-350N, 450A-450N) to contact the first aliquot; and

displacing the first aliquot and second aliquot through the contraction / expansion region (461, 462A-462D, 463A-463B, 481) to promote mixing between the first aliquot and the second aliquot.

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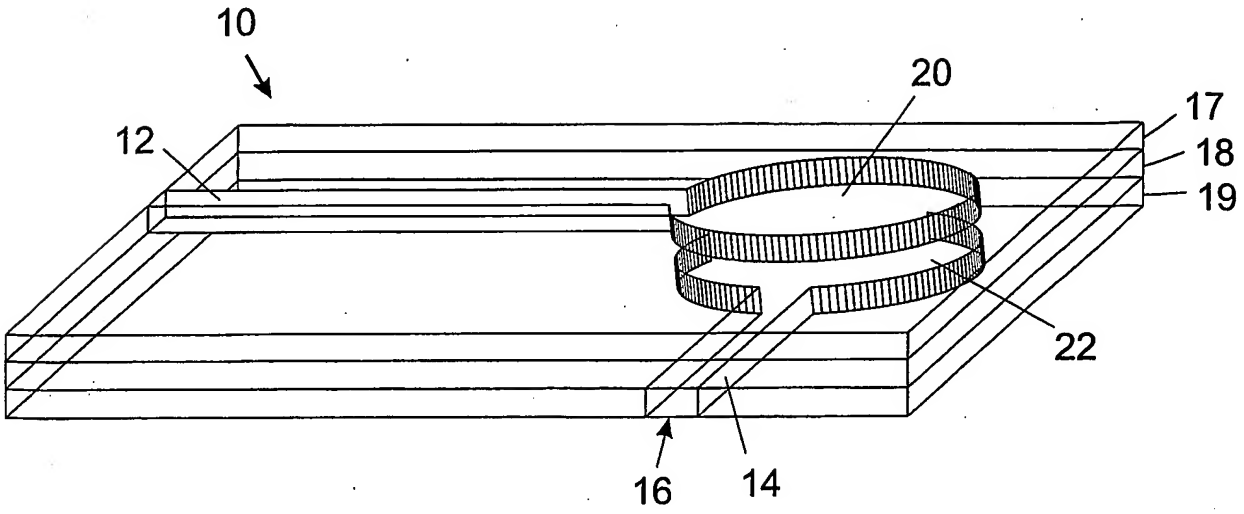
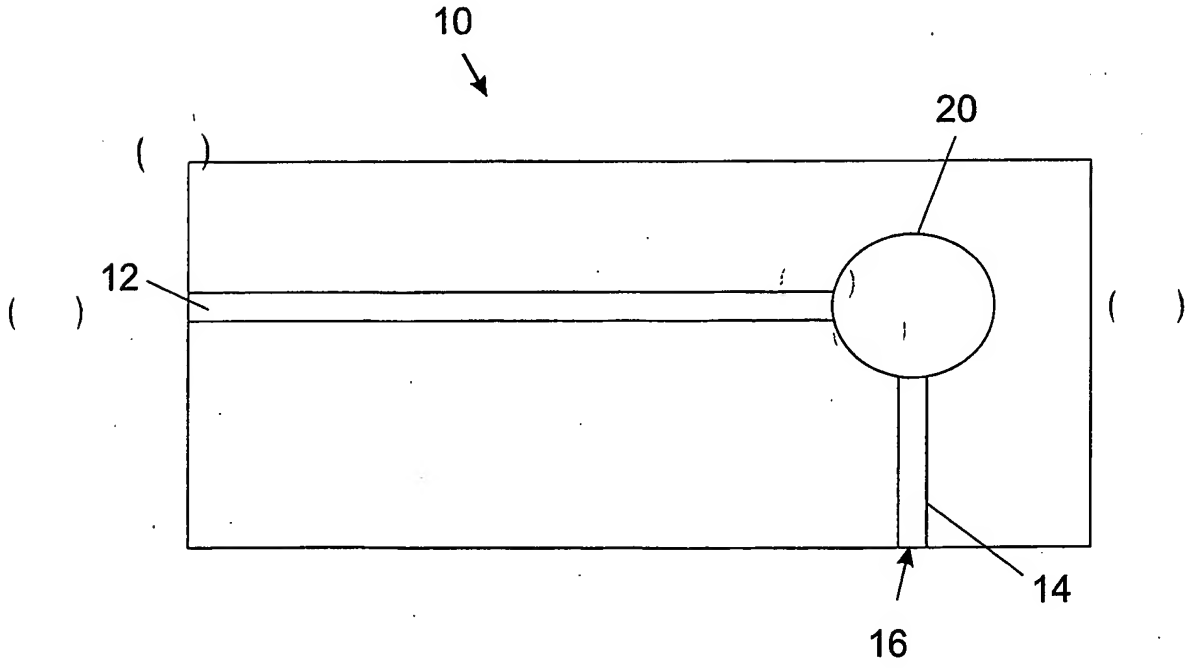


FIG._1A



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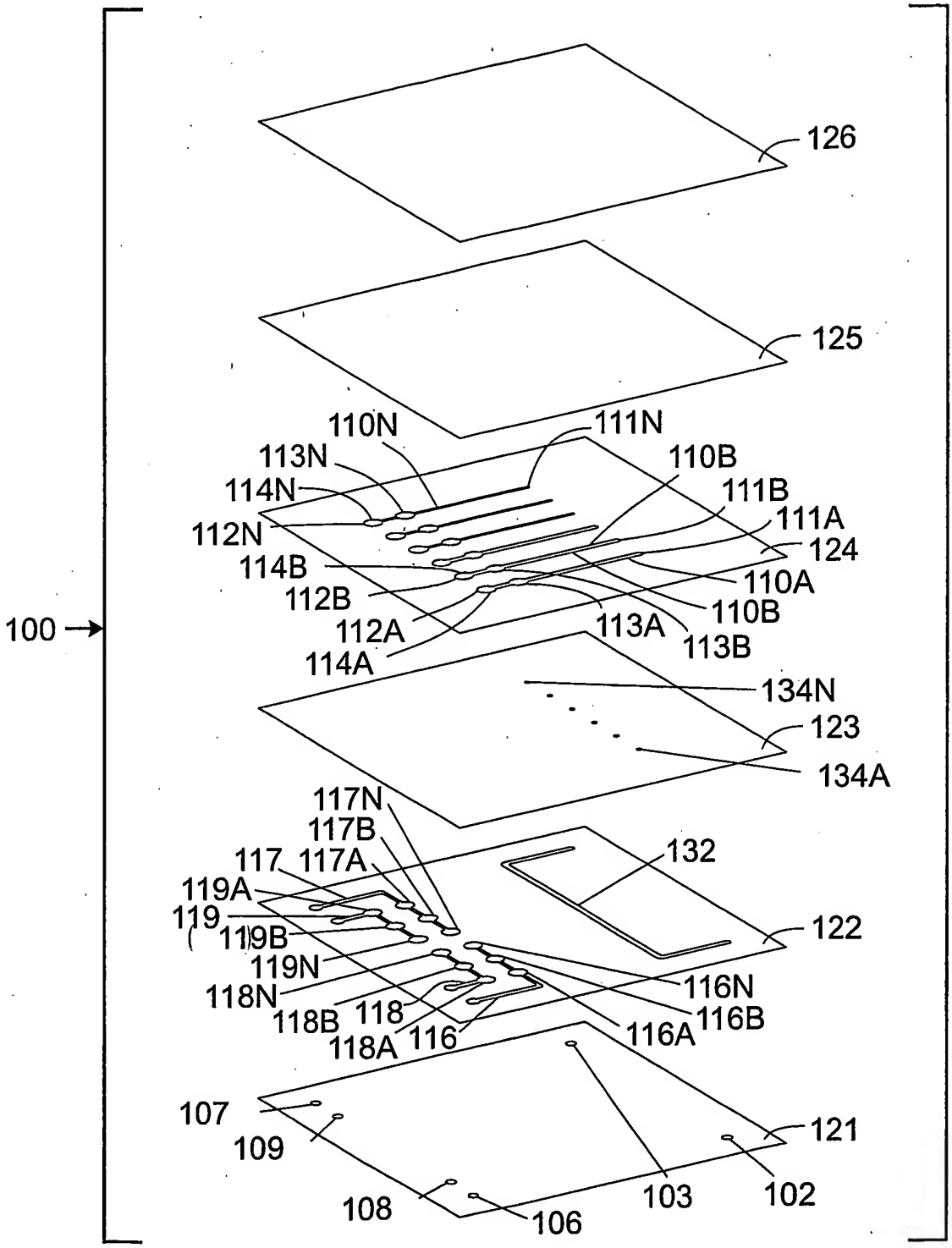
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FIG._2A

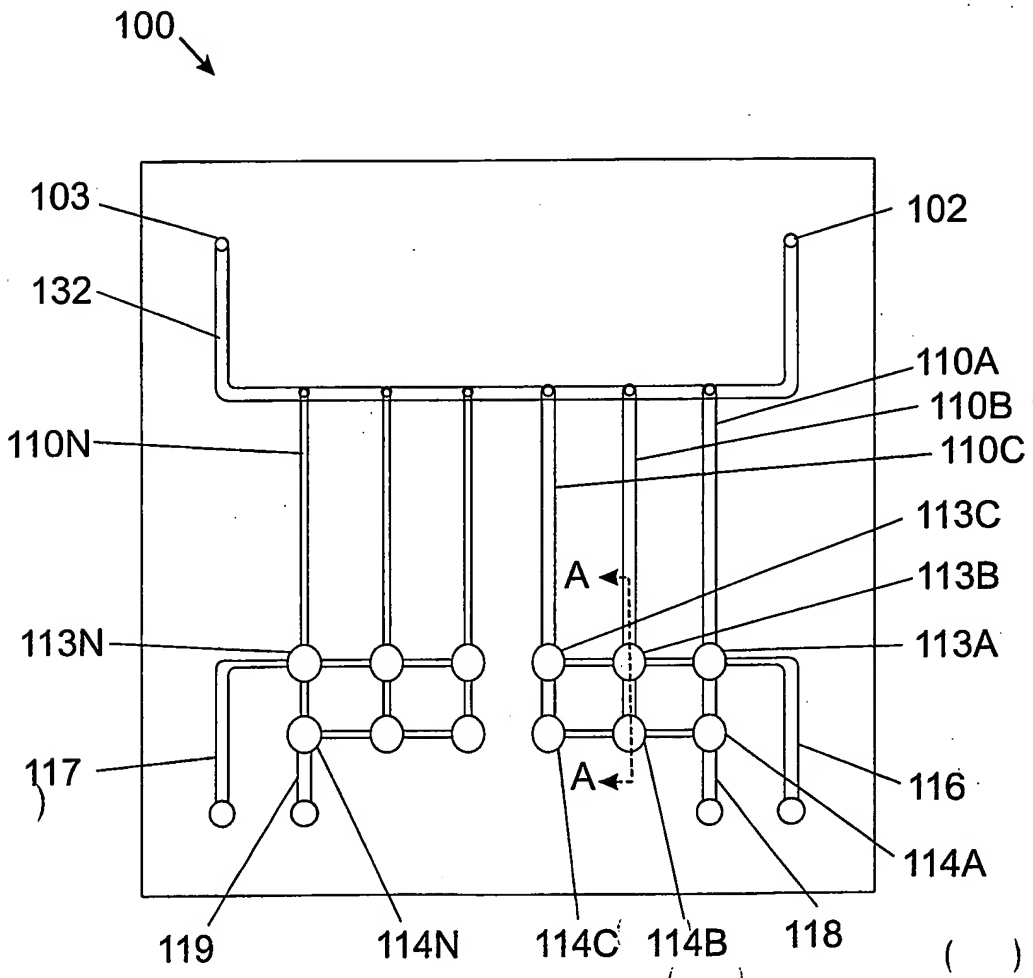
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FIG._2B

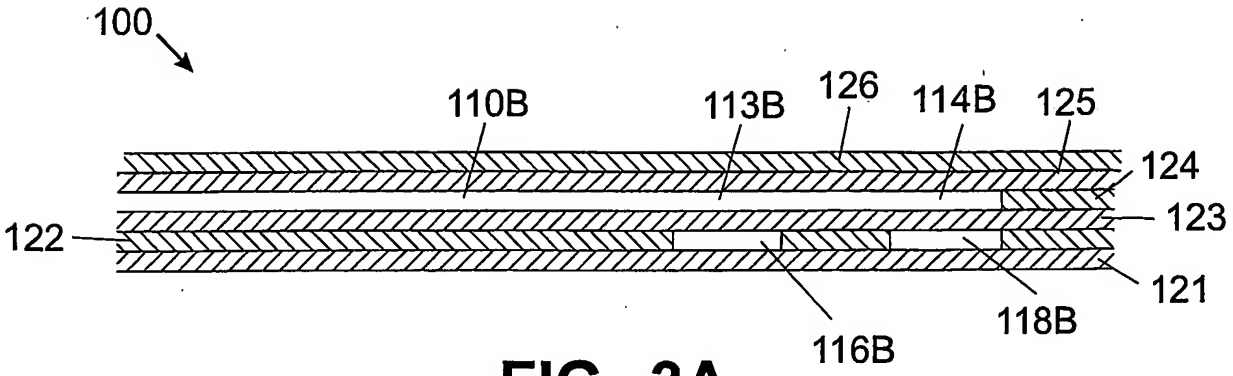

FIG._3A

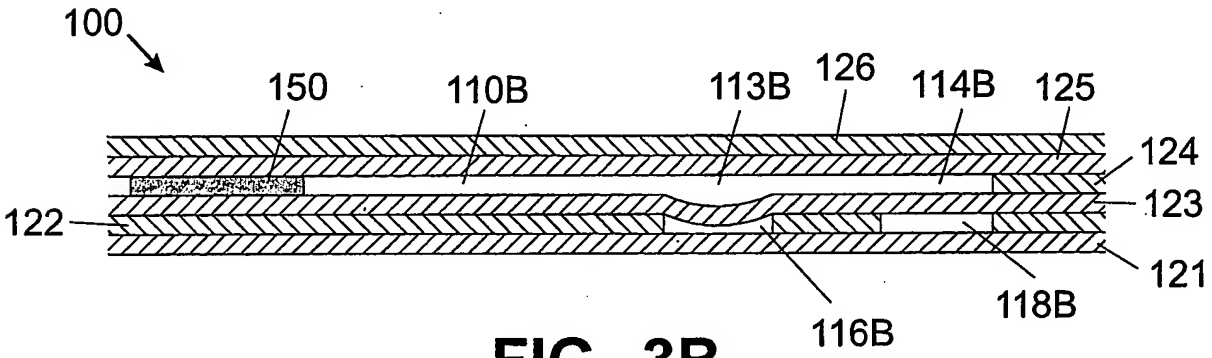

FIG._3B

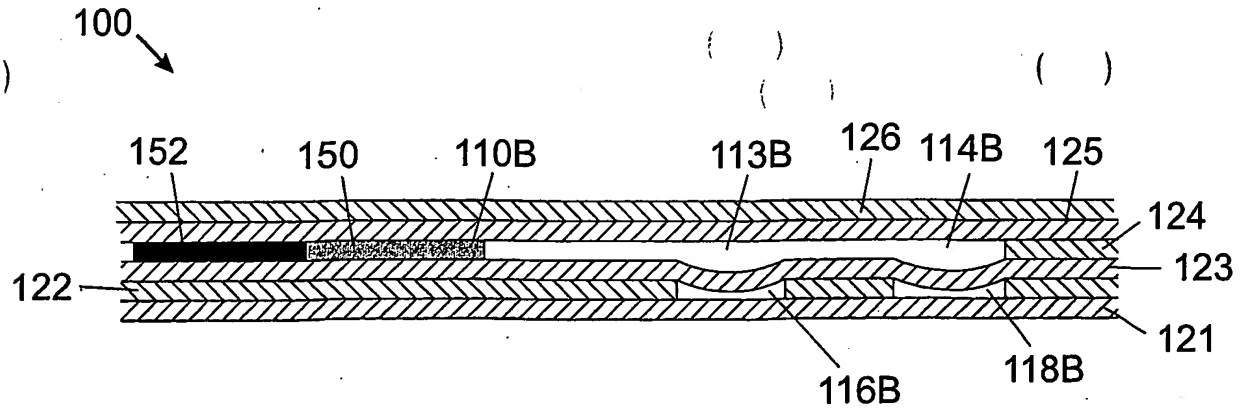

FIG._3C

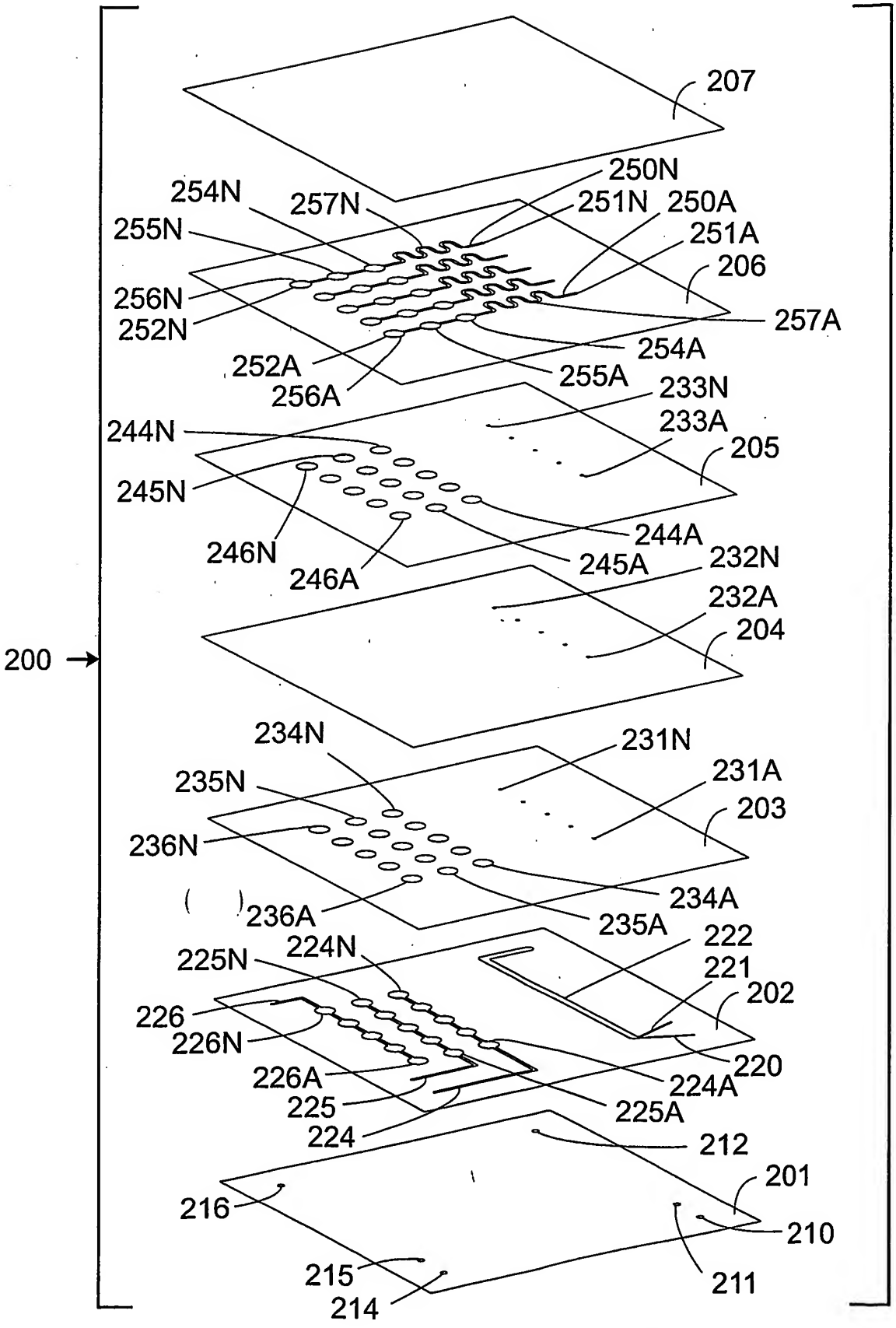
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FIG._4A

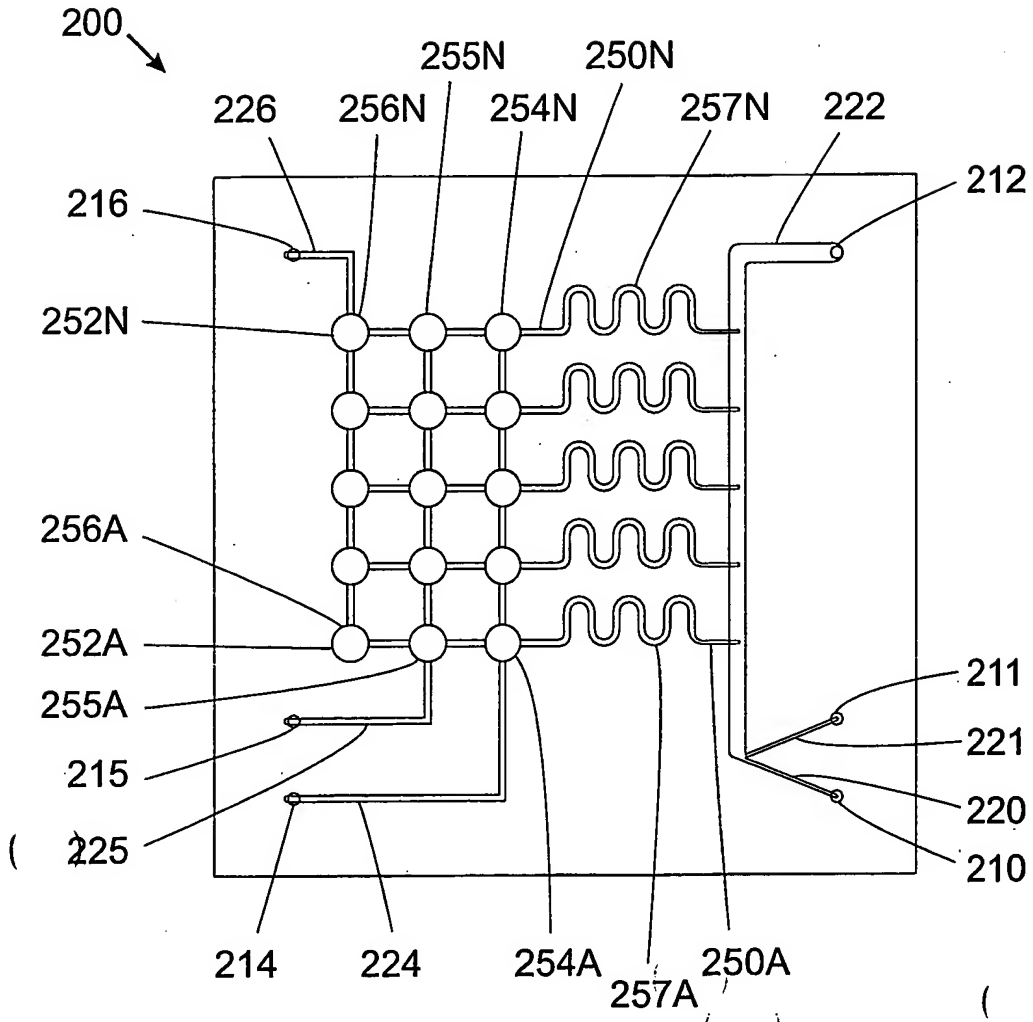
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FIG._4B

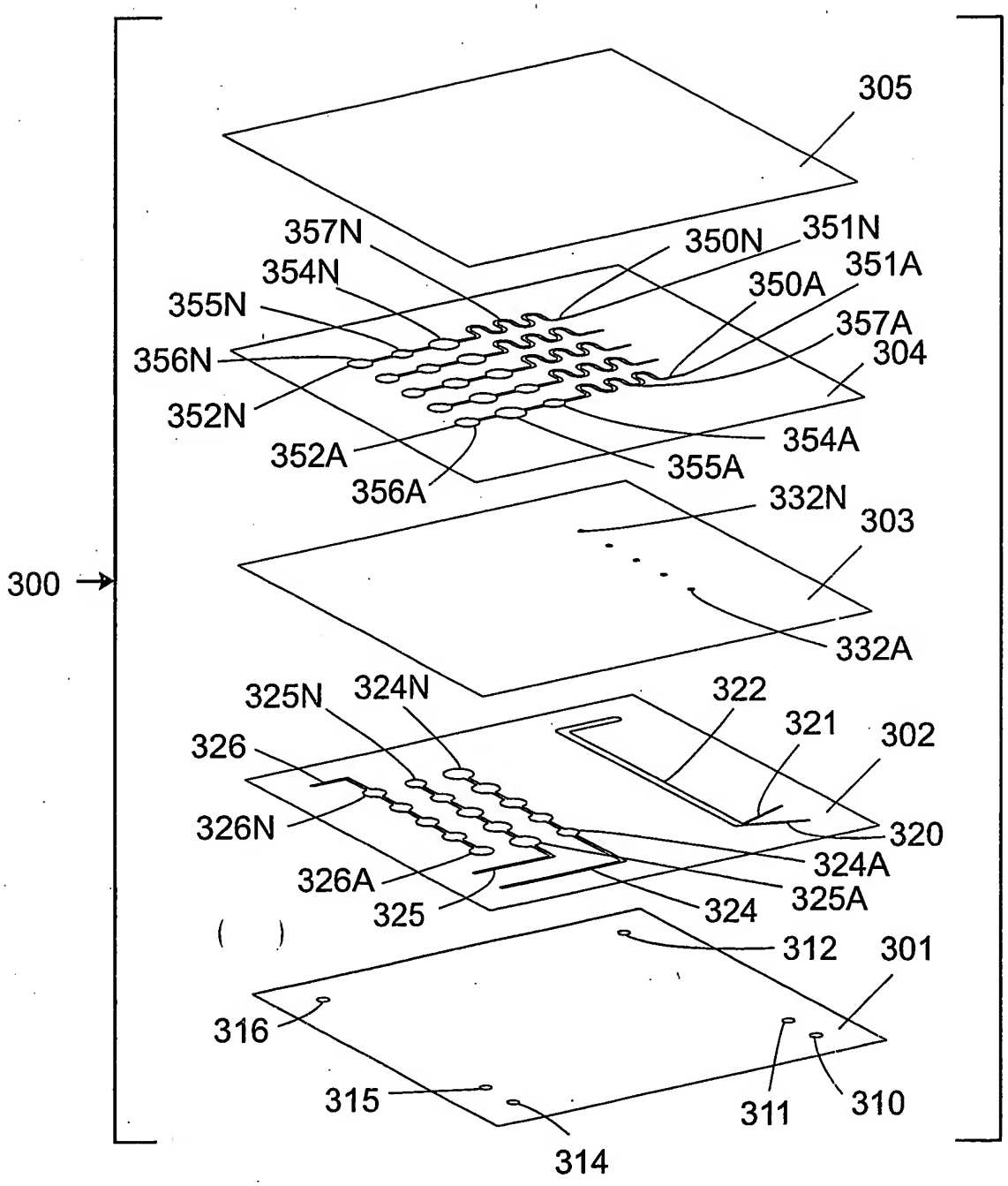
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FIG._5A

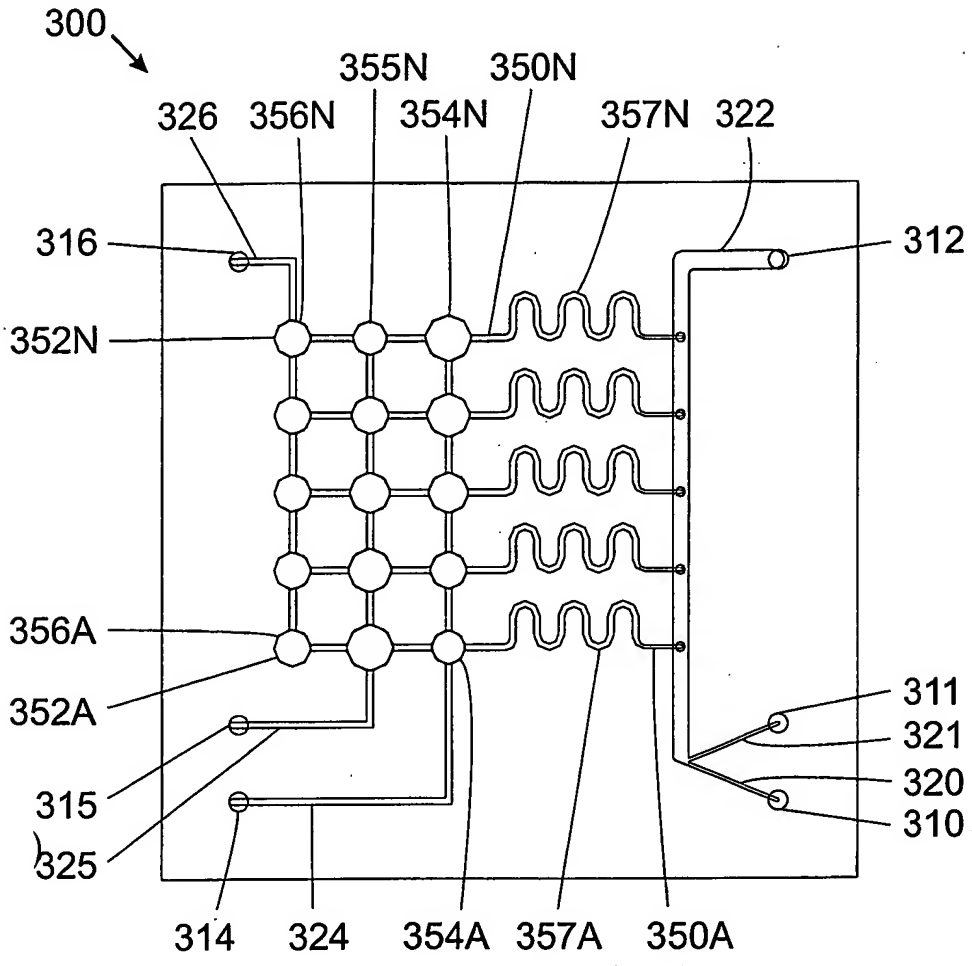
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FIG._5B

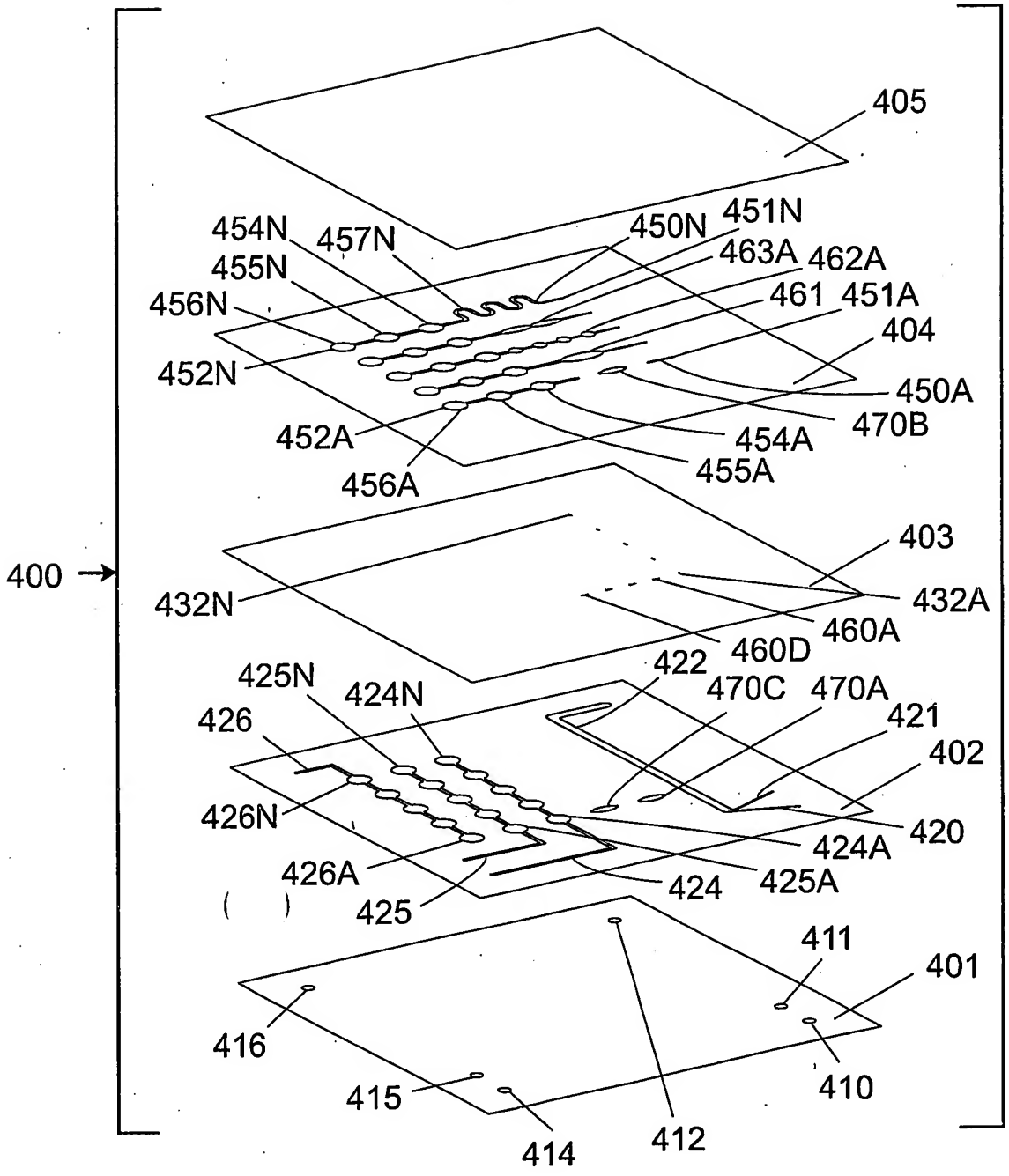
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FIG._6A

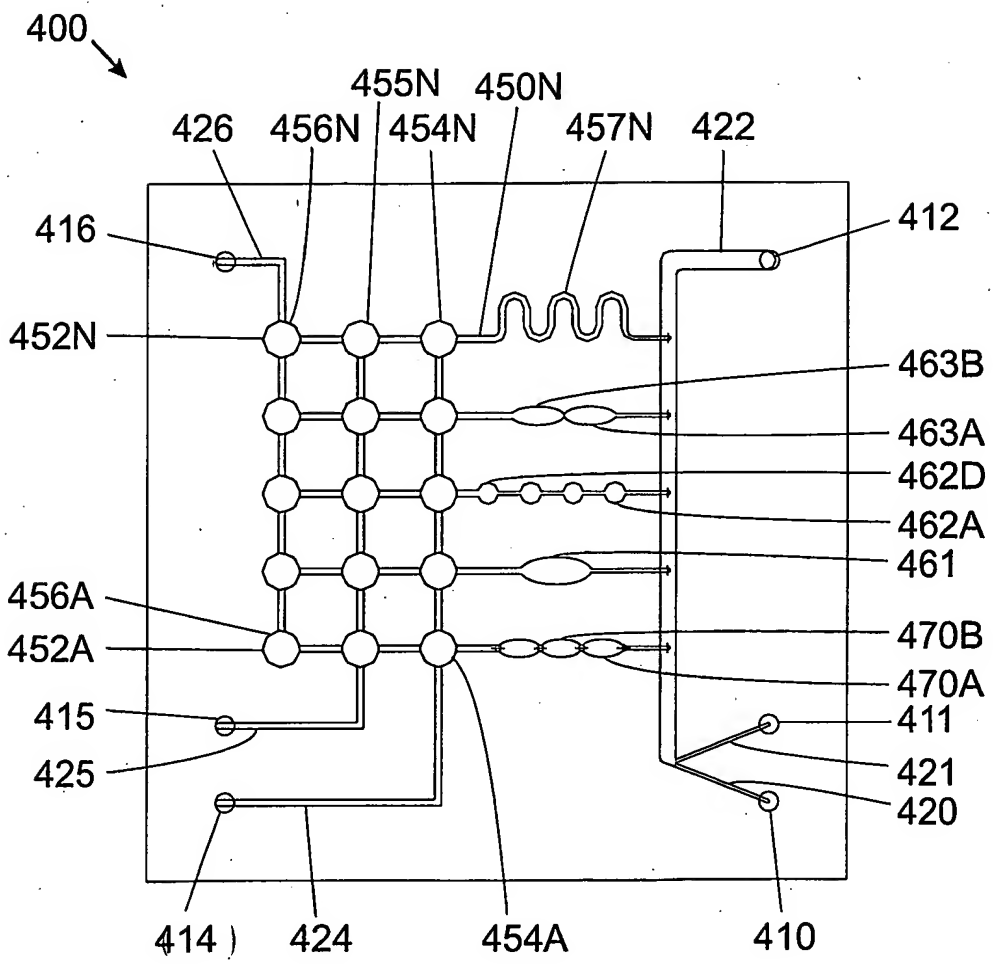
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FIG._6B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/21039

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	US 6 280 148 B1 (STEHR MANFRED ET AL) 28 August 2001 (2001-08-28) column 9, line 30 -column 10, line 34	1
A	DE 197 39 722 A (FREDRICH WOLFGANG ;MERKEL TOBIAS (DE); GRAEBER MICHAEL (DE); PAGEL) 1 April 1999 (1999-04-01) claim 1; figure 4	1
A	US 5 542 821 A (DUGAN JEFFREY S) 6 August 1996 (1996-08-06) abstract	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 2003	25/09/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Tragoustis, M

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